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Marek's Disease Virus Infection in the Eye: Chronological Study of the Lesions, Virus Replication, and Vaccine-Induced Protection

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SUMMARY. Marek's disease virus (MDV) infection in the eye was studied chronologically after inoculating 1-day-old chickens with a very virulent MDV strain, Md5. The ocular lesions could be classified as early lesions (6–11 days postinoculation [dpi]) and late lesions (26 and 56 dpi), based upon the location and severity of the lesions. The early lesions involved iris, ciliary body, and choroid layer, and were characterized by endothelial cell hypertrophy, vasculitis, and infiltration of lymphocytes (mainly CD8+), plasma cells, macrophages, and heterophils. Expression of early MDV-antigen pp38 in the cells infiltrating choroid layer was detected as early as 11 dpi. Late lesions consisted of severe lymphohistiocytic uveitis, keratitis, pectenitis, vitreitis, retinitis, and segmental to diffuse retinal necrosis. Cell infiltration included macrophages, granulocytes, plasma cells, and both CD4+ and CD8+ cells of various sizes. Expression of early MDV-antigen pp38 was readily found within the retina, uveal tract, and corneal epithelium. No expression of late-antigen gB or oncoprotein meq was detected in any of the eyes examined. A second experiment was conducted to study the effect of vaccination on the development of ocular lesions. Both HVT and CVI988 were able to protect against the development of early ocular lesions in chickens infected with very virulent plus strain MDV 648A. However, only CVI988 conferred complete protection against the development of late ocular lesions. HVT conferred partial protection, as it reduced the frequency and severity of the late ocular lesions. These results enhance our understanding of the nature and pattern of MDV infection in the eye.

RESUMEN. Infección en el ojo con el virus de la enfermedad de Marek: Estudio cronológico de lesiones, replicación viral y protección inducida mediante la vacunación.

La infección por el virus de la enfermedad de Marek se estudió cronológicamente luego de la inoculación de pollitos de un día de edad con una cepa muy virulenta del virus de la enfermedad de Marek denominada Md5. Basándose en la localización y severidad de las lesiones oculares estas pudieron ser clasificadas como lesiones tempranas (de seis a 11 días posteriores a la inoculación) y lesiones tardías (de 26 a 56 días posteriores a la inoculación). Las lesiones tempranas infectaron el iris, el cuerpo ciliar y la capa coroidea y se caracterizaron por hipertrofia de las células endoteliales, vasculitis e infiltración linfocitaria (principalmente linfocitos T CD8+), células plasmáticas, macrófagos y heterófilos. La expresión del antígeno temprano pp38 del virus de la enfermedad de Marek en las células infiltrando la capa coroidea se detectó desde el día 11 posterior a la inoculación,. Las lesiones tardías consistían en uveítis linfohistiocítica severa, queratitis, pectenitis, vitreítis, retinitis y necrosis retinal que varió de segmentada a difusa. La infiltración celular incluía macrófagos, granulocitos, células plasmáticas y células CD4+ y CD8+ de varios tamaños. La expresión del antígeno temprano pp38 del virus de la enfermedad de Marek fue muy evidente en la retina, tracto uveal y epitelio de la córnea. En ninguno de los ojos examinados se detectó la expresión del antígeno tardío gB o de la oncoproteína meq. Se realizó un segundo experimento para estudiar el efecto de la vacunación en el desarrollo de las lesiones oculares. Tanto el virus Herpes de pavo como el virus CVI988 fueron capaces de proteger contra el desarrollo de las lesiones oculares tempranas en pollos infectados con la cepa muy virulenta "plus" del virus de la enfermedad de Marek 648A. Sin embargo, solo el virus CVI988 confirió protección completa contra el desarrollo de las lesiones oculares tardías. El virus Herpes de pavo confirió protección parcial reduciendo la frecuencia y severidad de las lesiones oculares tardías. Estos resultados aumentan el conocimiento de la naturaleza y patrón de la infección en el ojo con el virus de la enfermedad de Marek.

Key words: Marek's diseases, eye, retina, pathogenesis

Abbreviations: ABC = avidin-biotin-peroxidase complex; CEF = chicken embryo fibroblasts; DEF = duck embryo fibroblasts; dpi = days postinoculation; H&E = hematoxylin and eosin; HSV = herpes simplex virus; HVT = herpesvirus of turkeys; MAb = monoclonal antibodies; MD = Marek's disease; MDV = Marek's disease virus; MHC = major histocompatibility complex; OCT = optimal cutting temperature; PN = peripheral neuropathy; S/C = subcutaneous route; SPAFAS = specific pathogen free; VZV = varicella-zoster virus

Marek's disease virus (MDV) is an alpha-herpesvirus that causes lymphomas, immunosuppression, and various neurologic syndromes in chickens (11,19,23,34). Ocular lesions consisting of pupil irregularities and loss of iris pigmentation (gray eye) have long been associated with Marek's disease (MD). The ocular tropism of MDV was demonstrated even before the identification of the virus. Between 1929 and 1943, a number of ocular lesions were described in chickens with presumed MD (26,28). According to these descriptions, the most prominent change involved the iris. After isolation of MDV in 1967 (11), a chronologic study of MDV-induced lesions in the eye was conducted (36) that confirmed the role of MDV in the development of lesions in the eye. This study also reported lymphoreticular proliferative lesions in choroid layer, ciliary body, cornea, and base of pecten (36). During the 1970s and 1980s, there were no reports of MDV-induced ocular lesions in commercial flocks, probably due to the introduction of vaccination in 1970 (12,42). However, in the early 1990s, several outbreaks of MD in vaccinated chickens were reported, outbreaks with increased virulence and unusual tropism for ocular tissues (14,37). The

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severity and extent of ocular lesions induced by new isolates of MDV were greatly increased, and ocular tropism was considered to be a biologic property of the emergent pathotype (14). Lesions reported by those authors included edema and predominantly mononuclear inflammatory cell infiltration within the cornea, uveal tract, retina, and pecten (14).

The pathogenesis of MDV-induced ocular lesions is still unknown. Considerable controversy still exists as to whether these lesions are inflammatory or neoplastic in nature. In addition, the role of MDV in the development of the ocular lesions is unclear. Ficken and coworkers observed intranuclear inclusion bodies in both mononuclear cells and retinal cells (14). However, it remains unknown if the viral infection of ocular tissues precedes the inflammatory cell infiltrates or if the infection is introduced by previously infected, infiltrating lymphocytes constituting part of the lesions.

The appearance of ocular lesions that lead to blindness in vaccinated chickens (5,14,37) is a serious problem in its own right. The continued increase in MDV virulence might lead to an increase in the incidence and severity of ocular lesions. Understanding the nature of these lesions and the effect of vaccination in their development will aid in their control. A better characterization of MDV-induced ocular lesions could also be of great use to further understand ocular lesions induced by human herpesviruses. Herpes simplex virus and varicella-zoster virus (VZV) induce a variety of ocular lesions including keratitis, keratoconjunctivitis, iridocyclitis, chorioretinitis, retinal necrosis, vitreitis, and optic neuritis. Intraocular inoculation of human herpesviruses into various rodents and nonhuman primates is used to study the pathogenesis of human herpesvirus-induced ocular diseases (1,25,29,38).

The objectives of this study were to chronologically examine MDV-induced ocular lesions, to characterize the nature and pattern of viral infection in the eye, and to evaluate the effect of vaccination on the development of MDV-induced ocular lesions.

MATERIALS AND METHODS

Chickens. In experiment 1, MD-susceptible F1 progeny (15 × 7) chickens were used from the Avian Disease and Oncology Laboratory line $15I_5$ males and line 7_1 females. All breeder chickens were free of antibodies to all three MDV serotypes; avian leukosis virus, reticuloendotheliosis virus, and various other poultry pathogens. In experiment 2, commercially available specific pathogen free (SPAFAS) chickens were used (Charles River SPAFAS, N. Franklin, CT).

Viruses. Oncogenic serotype 1 MDVs, very virulent strain Md5 at passage 8 in duck embryo fibroblasts (DEF; 41), and very virulent plus strain 648A at passage 10 in DEF (44) were used. Vaccine strains FC-126, at passage 10 in chicken embryo fibroblasts (CEF; 42) and CVI988 at passage 42 in CEF (32), were used.

Pathology. Lesions were evaluated both grossly and histologically. At necropsy, all chickens were examined for gross lesions in lymphoid organs (bursa of Fabricius, thymus, and spleen), peripheral nerves, viscera, and eyes. Eyes were immersed in Bouin's fixative for 24 hr and subsequently washed three times in 70% alcohol for 3 hr. Tissues were dehydrated in graded ethanol solutions, embedded in a low melting point (53–55 C) paraffin wax, sectioned at 5 μm, mounted on glass slides, and stained with hematoxylin and eosin (H&E). Lesions were evaluated subjectively and were scored from 0 (none) to 3 (severe).

Immunohistochemistry. An avidin-biotin-peroxidase complex (ABC; Vectastain ABC kit, Vector Laboratories, Burlingame, CA) was used for immunohistochemistry. Specifically, for the immunohistochemical staining of the meq antigen, the staining was amplified by using the tyramide signal-amplification reaction, following the manufacturer's instruction for the TSA Biotin System Kit (PerkinElmer Life Science, Boston, MA). To study MDV replication in the eye, the monoclonal antibodies (MAb) 1AN86.17 and H19 (35), against MDV gB and pp38, respectively, were used at a working dilution of 1:2000. Level of viral antigen expression was scored on a subjective scale of 0–3 based on the number of positive cells. Each slide was scored in a blind manner. The MAb 23B46 (22) against MDV-meq was used at a working dilution of 1:1000. Monoclonal antibodies used to study cell phenotype were purchased from Southern Biotech (Birmingham, AL). The MAb CT4 (7) against CD4 was used at a working dilution of 1:20. The MAb CT8 (7) against CD8 was used at a working dilution of 1:1. The MAb KUL01 (24) against macrophages was used at a working dilution of 1:5. The MAb CIa (13) against major histocompatibility complex (MHC) class II was used at a working dilution of 1:20.

Experimental design. Two experiments, in two replicates each, were conducted to chronologically study the development of ocular lesions (experiment 1) and to evaluate the effect of vaccination on the development of MDV-induced ocular lesions (experiment 2).

Chronological evaluation of ocular lesions (experiment 1). One-day-old 15 × 7 chickens were randomly divided into five groups of 20 chickens each and housed in Horsfall-Bauer isolation units (Plas Labs, Inc., Lansing, MI). Three groups were inoculated by the subcutaneous route (S/C) with 2000 PFU of Md5, and the other two groups served as uninoculated negative controls. Five chickens from each treatment group were euthanized and necropsied at 4, 6, 8, 11, and 26 day postinoculation (dpi). Both eyes were collected; one was placed in Bouin's solution for histopathologic evaluation and the other was placed in optimal cutting temperature (OCT) compound and frozen in liquid nitrogen for immunohistochemistry. In addition, eyes were collected from chickens that survived till the end of the experiment (8 weeks post inoculation) for histopathology and immunohistochemical evaluation.

Effect of vaccination on the development of ocular lesions (experiment 2). In experiment 2, 1-day-old SPAFAS chickens were randomly divided into seven groups of 20 chickens each and were housed in Horsfall-Bauer isolation units. Two groups were vaccinated S/C with 2000 PFU of HVT; two groups were vaccinated S/C with 2000 PFU of strain CVI988; and the other three groups served as uninoculated negative controls. One vaccinated group per each vaccine, as well as two nonvaccinated groups, were challenged S/C with 500 PFU of very virulent plus MDV strain 648A at 5 days of age. Five chickens from each treatment group were euthanized at 11 and 18 day after challenge, and eyes were collected in Bouin's solution for histopathologic analysis and in OCT compound for immunohistochemistry. Eyes were also collected from chickens that survived till the end of the experiment (8 wk postinoculation) or from moribund chickens that had to be euthanized for histopathology as well as for immunohistochemical evaluation.

Statistical analysis. Data were analyzed using the statistical program Statistica® (Statsoft, Tulsa, OK). A sign test was conducted to compare percentages between groups. To study differences in the intensity of lesions, a Kruskall–Wallis test was conducted. The level of significance considered was at $P < 0.05$.

RESULTS

Chronologic evaluation of ocular lesions (Figs. 1, 2; Table 1). The ocular lesions were classified as early lesions (6–11 dpi; Fig. 1) and late lesions (26 dpi and 56 dpi; Fig. 2) based on the severity and distribution of the lesions in the eye (Table 1).

The earliest (6 dpi) ocular lesions occurred within the iris and choroid layer. There was mild infiltration of lymphocytes and macrophages within the iris and ciliary body. There was vascular endothelial cell hypertrophy and mild perivascular infiltration of macrophages and lymphocytes, accompanied by mild edema within the choroid layer. By 11 dpi, there was moderate infiltration of lymphocytes, plasma cells, macrophages and occasional heterophils within the choroid layer (Fig. 1A,B), iris, and ciliary body (Fig. 1C,D). On 26 and 56 dpi, the choroid layer (Fig. 2A), iris

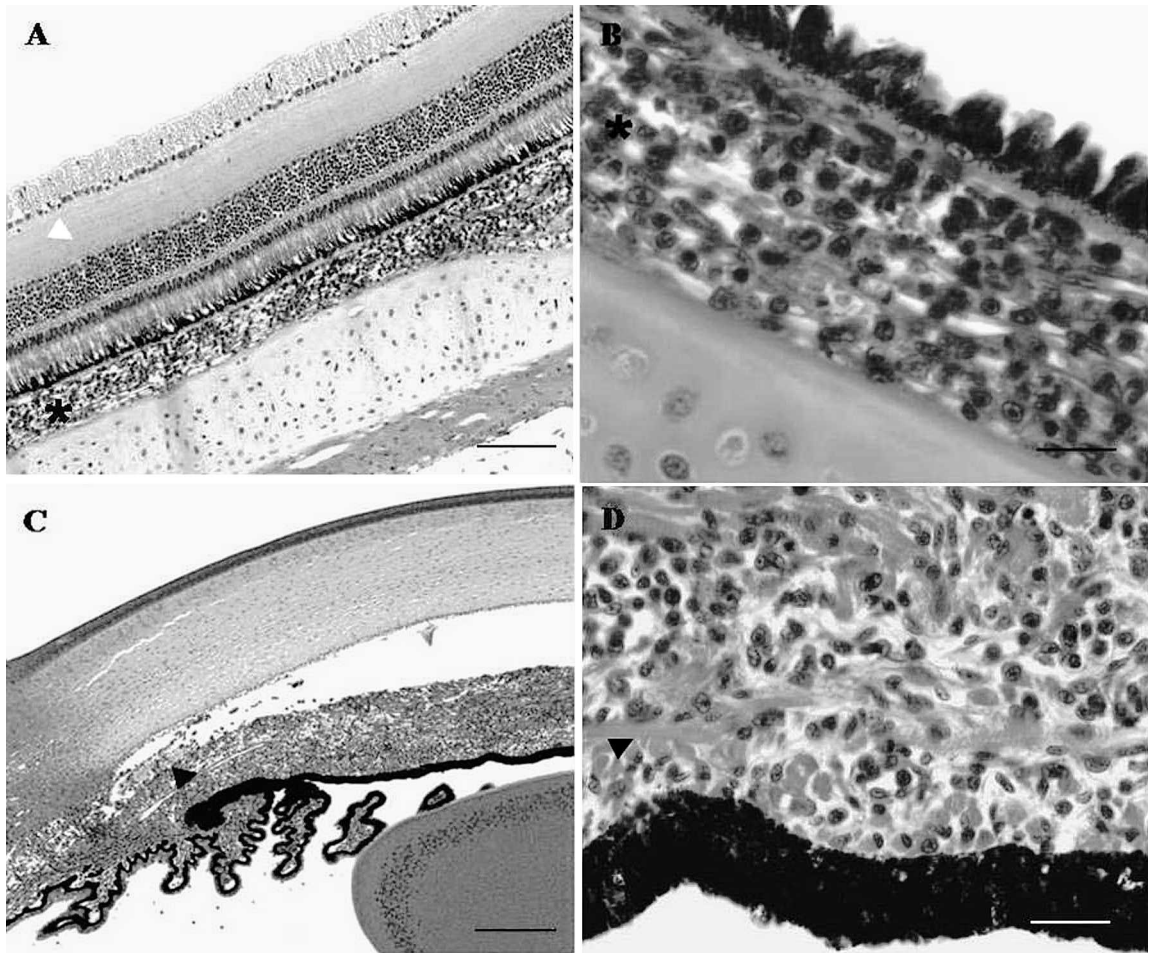

Fig. 1. Early lesions in the eyes of 15 × 7 chickens inoculated with MDV strain Md5 at hatch. (A) Choroid layer and retina at 11 dpi. Bar = 100 μm. (B) Choroid layer at 11 dpi. Bar = 33 μm. (C) Iris and cornea at 11 dpi. Bar = 125 μm. (D) Iris at 11 dpi. Bar = 50 μm. White arrowhead = retina; asterisk = choroid layer; black arrowhead = iris.

(Fig. 2B), and ciliary body were markedly expanded by lymphocytes, macrophages, and plasma cells. There were multifocal aggregates of macrophages and lymphocytes within the anterior chamber that, in some cases, deposited within the trabecular meshwork or were adhered to the corneal endothelium. In some chickens, the choroid layer was expanded by edema in addition to the mononuclear inflammatory cell infiltrate. The marked inflammatory cell infiltrate within the choroid layer invaded the retinal pigment epithelium (photoreceptor layer), as well as the outer and inner nuclear layers (outside in; Fig. 2A). Multifocally, there was retinal detachment with hypertrophy of the retinal pigment epithelium, along with accumulation of the inflammatory cell infiltrate and pale eosinophilic proteinaceous fluid within the subretinal spaces. The pecten was diffusely hypercellular and expanded by numerous lymphocytes, plasma cells, and large foamy macrophages (Fig. 2E,F). Numerous macrophages released into the vitreous humor from the pecten infiltrated the inner limiting membrane and deeper retinal layers, often destroying the laminar retinal architecture (inside out; Fig. 2A,C). Eventually, the mononuclear inflammatory cell infiltrate from the choroid layer and vitreous cell infiltrate (from pecten) diffusely effaced all the retinal layers and caused segmental to diffuse retinal necrosis, retinal detachment, or both (Fig. 2C). The earliest retinal lesions involved the peripheral retina, but later progressed to involve the central regions as well. At 26 and 56 dpi, eosinophilic intranuclear inclusion bodies were found in the retina (Fig. 2C). At 11 dpi, there was moderate infiltration of lymphocytes and plasma cells, and fewer macrophages were observed within the optic nerve. However, lesions within the optic nerve became more severe by 26 dpi, and they consisted of severe infiltration of large lymphoblasts intermixed with small lymphocytes and macrophages. Lesions within the cornea included moderate stromal infiltration of predominantly small lymphocytes, and had fewer plasma cells and macrophages, neovascularization, and mild edema causing disruption of the laminar collagen in the stroma (Fig. 2D). There was multifocal vacuolation and degeneration of the corneal epithelial cells, often accompanied by infiltration of small lymphocytes. The basement membrane was intact, with no evidence of ulceration. There was mild hypertrophy and occasional multifocal loss of the endothelial cells. There were rare intranuclear eosinophilic inclusions within the corneal epithelial cells.

Immunophenotypic characterization (Table 2; Fig. 3). Immunohistochemical characterization of the infiltrating cell population revealed that the relative incidence of each cell population slightly changed throughout the experiment (Table 2). At early stages (6–11 dpi), the mononuclear cell infiltrates within the choroid layer, ciliary body, and iris stained strongly positive for CD8 (Fig. 3A) and MHC-II, and mildly positive for macrophages and CD4. In addition, endothelial cells of infected chickens, but not of uninfected control chickens, expressed MHC-II as early as 6 dpi and throughout the experiment. Later (26–56 dpi), the infiltrating mononuclear cells

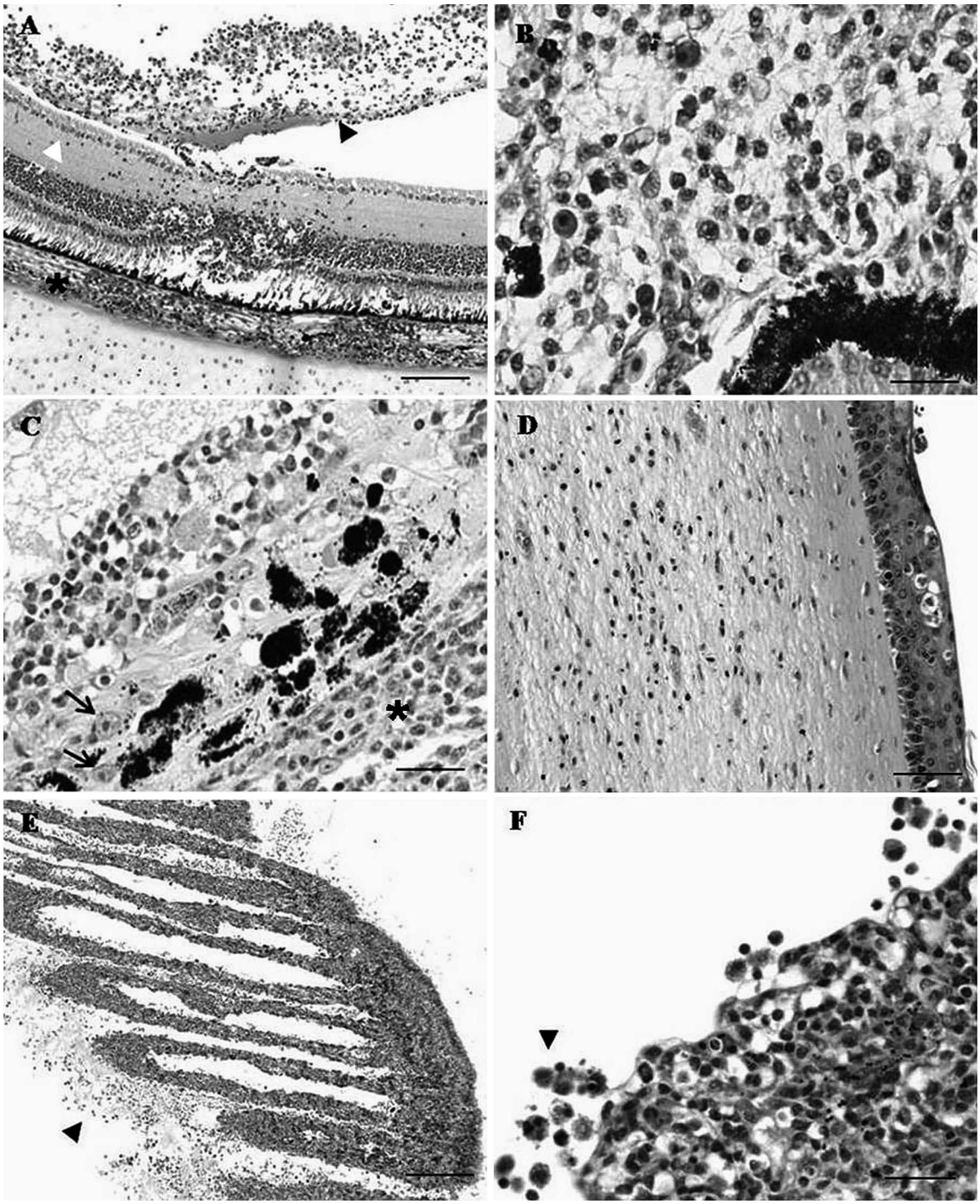

Fig. 2. Late lesions in the eyes of 15 × 7 chickens inoculated with MDV strain Md5 at hatch. (A) Choroid layer, retina, and vitreous at 26 dpi. Bar = 100 μm. (B) Iris at 56 dpi. Bar = 50 μm. (C) Choroid layer and retina at 56 dpi. Bar = 60 μm. (D) Cornea at 56 dpi. Bar = 90 μm. (E) Pecten and vitreous at 56 dpi. Bar = 175 μm. (F) Pecten and vitreous infiltrates at 56 dpi. Bar = 50 μm. White arrowhead = retina; asterisk = choroid layer; arrow = eosinophilic inclusion bodies; black arrowhead = mononuclear cell infiltrates in vitreous.

within the uveal tract and retina were mildly positive for CD4 (Fig. 3B), CD8 (Fig. 3C), and macrophages (Fig. 3D), and the cells remained strongly positive for MHC-II (Fig. 3E).

The infiltrating cells within the early lesions were negative for gB and meq, but scattered cells were positive for pp38 antigen within the choroid layer as early as 11 dpi. Within the late lesions (26 and 56 dpi), the infiltrating cells were negative for gB and meq, but there were numerous cells positive for pp38 antigen within the uveal tract, retina, and cornea (Fig. 3F). Corneal epithelium was multi-focally strongly positive for viral antigen pp38.

Effect of vaccination on the development of ocular lesions (Tables 3, 4). Lesions induced by 648A in SPF chickens were very similar to those induced by Md5 in 15 × 7 chickens (Tables 1, 3). At 11 dpi, there was moderate infiltration of lymphocytes, plasma cells, macrophages, and occasional heterophils within the choroid layer and iris (early lesions). On 18 dpi and later, lesions were found

Table 1. Chronologic evaluation of lesions in the eye of 15 × 7 chickens inoculated at hatch with very virulent Marek's disease virus strain Md5.

Lesions	dpi	No. chickens	Cornea	Iris	Choroid layer	Retina	Optic nerve	Pecten	Vitreous humor
Early[A]	4	10	0% (0+)[B]	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
	6	10	0% (0+)	11.1% (0.5+)	55.6% (0.5+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
	8	10	0% (0+)	0% (0+)	20% (0.5+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
	11	10	0% (0+)	60% (1+)	60% (2+)	0% (0+)	40% (1+)	0% (0+)	0% (0+)
Late	26	10	40% (1+)	60% (1.5+)	60% (2+)	40% (1+)	60% (1+)	60% (2+)	60% (1+)
	56	18	83% (2.5+)	100% (2.5+)	100% (3+)	94.4% (3+)	72.2% (1+)[C]	88.9% (2+)	83% (2.5+)

[A]Ocular lesions were classified into early and late, based on distribution of the lesions and severity of the infiltrates. Early lesions were characterized by mild to moderate mononuclear infiltration in iris and choroid layer. Late lesions were characterized by severe mononuclear infiltration in cornea, iris, choroid layer, retina, pecten, and vitreous humor. In addition, late lesions were characterized by severe retinal degeneration and changes within the corneal epithelium.

[B]Results from two replicates are presented in this table. Results are presented as percentage of animals that developed lesions. Results in brackets = severity of the lesions on a subjective scale of 0–3.

[C]Lesions in the optic nerve at 56 dpi differed from lesions described in other structures of the eye. Optic nerve lesions were characterized by infiltration of large lymphoblasts intermixed with small lymphocytes and macrophages resembling type A lesions (3).

not only in the choroid layer and iris, but also in the cornea, retina, optic nerve, pectin, and vitreous humor (late lesions).

Chickens vaccinated with either HVT or CVI988, and then challenged with 648A, developed very mild early lesions (11 dpi) that, statistically, were significantly different than those developed by unvaccinated chickens challenged with 648A (Tables 3, 4). Similar mild lesions were found in chickens vaccinated with CVI988 that were not challenged with 648A. Chickens vaccinated with HVT but not challenged with 648A, however, did not develop any early lesions.

None of the chickens vaccinated with CVI988 and challenged with 648A, or vaccinated with either CVI988 or HVT and unchallenged, developed late lesions. Some of the chickens, vaccinated with HVT and challenged with 648A, developed moderate lesions in the iris (86%), cornea (43%), choroid layer (29%), retina (43%), pecten (43%), and vitreous humor (43%) at 56 dpi. The percentage of HVT-vaccinated and challenged chickens with lesions at 56 dpi, and the intensity of those lesions, statistically were significantly lower than the lesions in unvaccinated and challenged chickens at 31 dpi (data not shown). Comparison between those two groups at 56 dpi was not possible because none of the unvaccinated and challenged chickens survived until 56 dpi.

DISCUSSION

The results of this study indicated that MDV infection in chickens induces severe ocular lesions. These lesions could be categorized into early and late, based on the location and severity. The early lesions were characterized by mild to moderate lymphohistiocytic uveitis. The late lesions were characterized by severe lymphohistiocytic uveitis, keratitis, pectenitis, vitreitis, retinitis, and segmental to diffuse retinal necrosis. The evolution of MDV-induced lesions in the eye resembles those previously described in the brain (16). In both cases, lesions start very early after infection (6 dpi) and consist of hypertrophy of endothelial cells and infiltration of CD8+ lymphocytes, CD4+ lymphocytes, and macrophages. What is remarkable, however, was the presence of plasma cells and granulocytes in the ocular lesions; these are not commonly present in brain lesions (9,16,18,19). The development and nature of the late lesions in the eye is still unclear. In the brain, late lesions are characterized by infiltration of large CD4+ lymphoblasts, with minimal or no infiltration of CD8+ lymphocytes, macrophages, plasma cells, or granulocytes (9,16,18,19). In the eye, however, infiltration of CD4+ cells was scattered, and they were intermixed with CD8+ cells, macrophages, plasma cells, and granulocytes. In addition, several chickens showed moderate edema in the choroid layer. The presence of edema, plasma cells, and macrophages are all characteristics of the type B lesions induced by MDV in the peripheral nerves (30). However, type B lesions seem to occur as an immune response against neoplastic cells invading the nerves, and they occur later in the pathogenesis of the disease (21,31). Similar types of infiltrates have also been described in the nerves of chickens that were inoculated with MDV as adults and never developed tumors (41). In addition, type B-like lesions have been reported in the nerves of chickens that are inoculated with a mutant MDV lacking the gene pp38 (rMd5Δpp38) (17). The infiltrating cells in lesions induced by rMd5Δpp38 underwent apoptosis, and the development of gross tumors was severely impaired. Finally, type B-like lesions in the nerves have been described in layers with peripheral neuropathy (PN) syndrome (2). PN appears to be an immune-mediated syndrome unrelated to MDV infection, although some MDV vaccines have been implicated in its pathogenesis (2). The nature of type B-like lesions is unclear, but it seems possible that they have an immune-mediated mechanism. This being the case, the late lesions observed in the eye of chickens inoculated with MDV might share this immune-mediated mechanism, and further studies are warranted.

The detection of MDV antigens in at least a subpopulation of infiltrating cells in the eye supports a productive infection. Early protein pp38 was detected in both early and late lesions. However, late protein gB and the oncoprotein meq were not detected at any time-point during the study. Expression of pp38, but not of gB, indicates that MDV establishes an abortive infection in the eye. This finding has also been reported in the brain (8,16) and in feather pulp (10). Mechanisms of the disruption of cytolytic infection, and its

Table 2. Summary of Marek's disease-induced ocular lesions, immunophenotype and viral antigen expression of the infiltrating cell population.

Characteristics	Early lesions (6–11 dpi)	Late lesions (26 and 56 dpi)
Location	Uveal tract	Uveal tract, cornea, retina[A]
Cell phenotype infiltrates	CD4+, CD8++, MΦ+, MHC-II++	CD4+, CD8+, MΦ+, MHC-II++
Viral antigen expression	pp38+, gB−, meq−	pp38++, gB−, meq−

[A]Optic nerve, pectin, and vitreous humor were not available in the sample stained by immunohistochemistry; therefore, data are not provided.

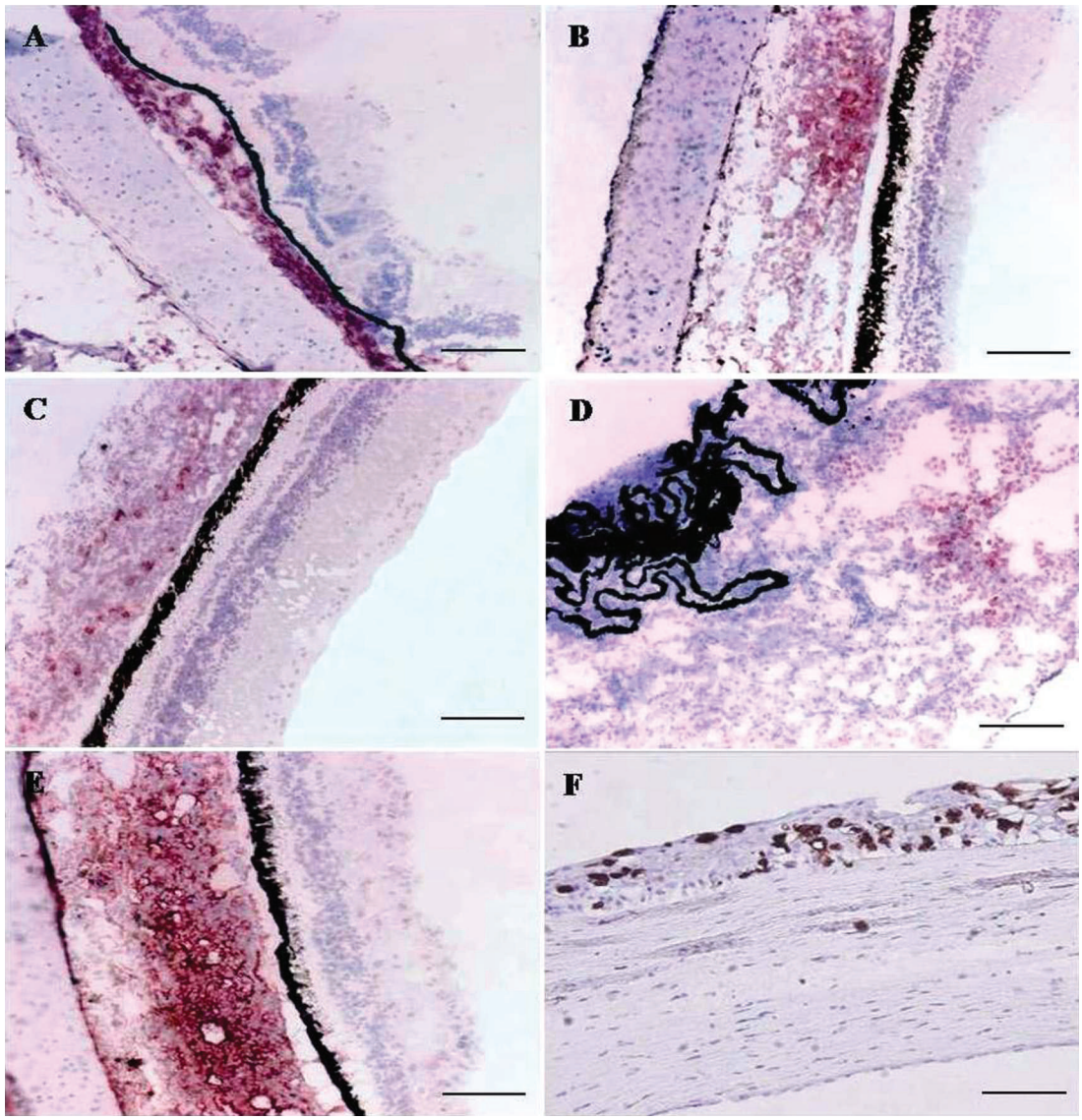

Fig. 3. Immunophenotype of cells infiltrating the eyes of 15 × 7 chickens inoculated with strain Md5 at hatch. (A) Mononuclear cells infiltrating the choroid layer were diffusely strongly positive with CT8 (CD8) at 11 dpi, immunohistochemical stain (substrate vector red [VR]). Bar = 100 μm. (B) Mononuclear cell infiltrating the choroid layer were focally extensively positive with CT4 (CD4) at 26 dpi, immunohistochemical stain (substrate VR). Bar = 100 μm. (C) Mononuclear cells infiltrating the choroid layer were scattered positive with CT8 (CD8) at 26 dpi, immunohistochemical stain (substrate VR). Bar = 100 μm. (D) Mononuclear cells infiltrating the iris were extensively positive with KUL01 (macrophages) at 11 dpi, immunohistochemical stain (substrate VR). Bar = 75 μm. (E) Diffusely, the mononuclear cells and endothelial cells within the choroid layer were strongly positive with CIa (MHC-II) at 26 dpi, immunohistochemical stain (substrate VR). Bar = 100 μm. (F) Corneal epithelium stained with H19 (pp38) at 56 dpi, immunohistochemical stain (substrate diaminobenzidine). Bar = 50 μm.

relevance in the pathogenesis of MDV infection in the eye, are poorly understood.

The lack of expression of meq in the late lesions contrasts with the strong meq expression in MDV-induced lymphomas (15,16,33). This finding, together with the reduced number of CD4+ lymphocytes in late lesions and the moderate infiltrates of macrophages and plasma cells, confirms that the MDV-induced late lesions in the eye have marked differences with the neoplastic lymphoproliferative lesions reported in other locations (31).

Previous descriptions of MDV-induced ocular lesions have focused on the development of late lesions (14,36). Smith and coworkers conducted a chronologic study of the ocular lesions induced by virulent strain GA in the susceptible line of chicken P bearing maternal antibodies (36). Those authors found that the earliest ocular lesions were induced at 18 dpi. In this work, we showed that in chickens without maternal antibodies, very virulent strain Md5 induced ocular lesions as early as 6 dpi. Smith and coworkers described severe lesions in the choroid layer, iris, ciliary bodies, and corneal epithelium (36). Similar lesions were also reported by Spencer and coworkers (37). However, none of those studies reported lesions in the pecten, vitreous humor, and retina (36,37). In our work, the pecten, vitreous humor, and retina were severely affected in most chickens. Lesions in the retina and pectin, similar to the late lesions described in this work, were reported previously (14). Ficken and coworkers attributed the severity of ocular lesions in their study to an increased virulence of MDV strains used (14). In their study, commercial egg-type breeder chickens were inoculated with several MDV strains of varying virulence, and ocular lesions were studied at 8 wk after inoculation. Interestingly, the percentage of chickens that developed ocular

Table 3. Effect of various vaccines on the development of ocular lesions induced by very virulent plus Marek's disease virus strain 648A in specific pathogen free chickens inoculated at hatch.

Group	dpi[A]	No. chickens	Cornea	Iris	Choroid layer	Retina	Optic nerve	Pecten	Vitreous humor
648A	11	10	0% (0+)[B]	100% (1+)	100% (2+)	0% (0+)	20% (0.5+)	0% (0+)	0% (0+)
	18	10	60% (1+)	100% (1.5+)	100% (2+)	100% (1+)	80% (1+)	100% (2+)	100% (2+)
	31	7	100% (2+)	100% (2+)	100% (2.5+)	86% (2+)	100% (2+)	100% (2+)	100% (1.5+)
HVT/648A[C]	11	10	0% (0+)	40% (0.5+)	80% (0.5)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
	18	10	0% (0+)	20% (0.5+)	40% (0.5)	0% (0+)	20% (0.5+)	0% (0+)	0% (0+)
	56	10	43% (1+)	86% (1+)	29% (0.5+)	43% (1+)	0% (0+)	43% (1+)	43% (1.5+)
CVI988/648[C]	11	10	0% (0+)	40% (0.5+)	40% (1+)	0% (0+)	20% (0.5+)	0% (0+)	0% (0+)
	18	10	0% (0+)	20% (0.5+)	0% (0+)	0% (0+)	20% (0.5+)	0% (0+)	0% (0+)
	56	10	0% (0+)	33% (0.5)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
CVI988	11	10	0% (0+)	67% (0.5+)	50% (0.5+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
	18	10	0% (0+)	33% (0.5+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
	56	10	0% (0+)	29% (0.5+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
HVT	11	10	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
	18	10	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
	56	10	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
Negative control	11	10	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
	18	10	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
	31	6	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)
	56	4	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)	0% (0+)

[A]Eyes were collected at 11, 18, and 56 dpi. Chickens inoculated with 648A showed severe neurologic clinical signs by 31 dpi and had to be euthanatized. None of the chickens of this group survived until 56 dpi. A few chickens from the uninoculated control group were euthanatized at 31 dpi as a control for that day.

[B]Results from two replicates are presented in this table. Results are presented as percentage of animals that developed lesions; results in brackets = severity of the lesions on a subjective scale of 0–3.

[C]Vaccination with herpesvirus of turkeys confers protection against early events of the pathogenesis of 648A (early cytolytic infection and transient paralysis), but does not protect against late events (development of tumors induced by 648A; 40); Vaccination with CVI988 confers protection against both early events of the pathogenesis of 648A (early cytolytic infection and transient paralysis) and late events (neoplasia).

lesions when inoculated with strain Md5 was very low, compared with the results of our work (14). The presence of maternal antibodies, and the different chicken genetic line used in that study, might have contributed to those differences. In addition, our results did not show major differences in the type of lesions induced by a very virulent MDV (Md5) and a very virulent plus MDV (648A). However, we did not conduct both experiments at the same time, and two different chicken strains were used, so a direct comparison was not possible.

Pecten seems to play a major role in the development of the late lesions described in this paper. There was a correlation between the severity of lesions in the pecten, the vitreous humor, and the retina

Table 4. Statistical analysis of the effect of various vaccines on the development of ocular lesions induced by very virulent plus Marek's disease virus strain 648A in specific pathogen free chickens.[A]

			Cornea[B]		Iris[B]		Choroid layer[B]		Retina[B]		Optic nerve[B]		Pecten[B]		Vitreous humor[B]	
dpi	Group	No. chickens	%	I	%	I	%	I	%	I	%	I	%	I	%	I
11	648A	10	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	HVT[C]/648A	10	a	a	b	ab	ab	b	a	a	a	a	a	a	a	a
	CVI988/648A	10	a	a	b	ab	b	ab	a	a	a	a	a	a	a	a
	CVI988	10	a	a	ab	ab	b	b	a	a	a	a	a	a	a	a
	HVT	10	a	a	c	b	c	b	a	a	a	a	a	a	a	a
	None	10	a	a	c	b	c	b	a	a	a	a	a	a	a	a
18	648A	10	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	HVT/648A	10	b	b	b	b	b	b	b	b	b	a	b	b	b	b
	CVI988/648A	10	b	b	b	b	c	b	b	b	b	a	b	b	b	b
	CVI988	10	b	b	b	b	c	b	b	b	b	a	b	b	b	b
	HVT	10	b	b	b	b	c	b	b	b	b	a	b	b	b	b
	None	10	b	b	b	b	c	b	b	b	b	a	b	b	b	b
31	648A	7	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	None	6	b	b	b	b	b	b	b	b	b	b	b	b	b	b
56	HVT/648A	10	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	CVI988/648A	10	b	b	b	b	b	a	b	b	a	a	b	b	b	b
	CVI988	10	b	b	b	b	b	a	b	b	a	a	b	b	b	b
	HVT	10	b	b	b	b	c	b	b	b	b	a	b	b	b	b
	None	10	b	b	c	b	b	a	b	b	a	a	b	b	b	b

[A]Statistical analysis was conducted, by days, between treatment groups. To study differences in the percentage (%) of chickens with lesions, a *t*-test to compare percentages was used. To study differences in the intensity (I) of the lesions, a Kruskall–Wallis test was used. $P < 0.05$.

[B]Different letters indicate that statistically significant differences were found among treatment groups.

[C]Herpesvirus of turkeys.

(data not shown). Mononuclear inflammatory cell infiltrates, predominantly macrophages, released from the pecten populated the vitreous humor. These inflammatory cell infiltrates invaded the retinal inner-limiting membrane and infiltrated all retinal layers, and caused degeneration and necrosis with segmental to diffuse retinal detachment. The role of pecten in the avian eye is still under discussion, but it is believed to play a role in retinal nourishment and in controlling vitreous pH (20).

Vaccination with HVT and CVI988 protected against the development of early lesions induced by 648A. It has been previously reported that vaccination with any serotype of MDV is able to protect against the early events in the pathogenesis of MDV related to MDV replication (early cytolytic infection and transient paralysis) (6,18). Vaccination with CVI988 also protected against the development of late lesions. CVI988 has been demonstrated to protect against the later events in the pathogenesis of 648A that result in neoplasia. Vaccination with HVT delayed the onset of late lesions and reduced the frequency and severity, but did not confer total protection. HVT does not confer protection against the development of tumors induced by 648A (40). This indicates that, even though late ocular lesions have remarkable differences with neoplastic lesions in other locations, they might have some neoplastic component. Nonetheless, the percentage of HVT-vaccinated chickens that develop tumors in viscera and nerves, when challenged with 648A, is generally higher than the percentage of chickens that developed late ocular lesions in our study, and the lesions were as severe as in the unvaccinated chickens (40). Ours results show that late lesions can develop in the absence of early lesions (chickens vaccinated with HVT and challenged with 648A developed late lesions but not early lesions). This finding suggests that vaccine-induced protection against early lesions and late lesions follow different mechanisms. Both early and late MDV-induced ocular lesions seem to have an inflammatory component. However, it is currently unknown if the late ocular lesions could be a consequence of an immune response against neoplastic cells, as in the type-B nerve lesions.

Various herpesviruses induce ocular lesions in humans and other animals. Ocular lesions induced by human herpesviruses include keratitis, keratoconjunctivitis, iridocyclitis, chorioretinitis, retinal necrosis, and vitreitis. The pathogenesis of human herpesviruses in the eye is not well understood, mainly because of the lack of a satisfactory animal model. Intraocular inoculation of human herpesviruses into various rodents and nonhuman primates is used to study the pathogenesis of human herpesvirus-induced ocular disease. Some of the models include examination of the uninoculated contralateral eye following uniocular anterior chamber inoculation of herpes simplex virus (HSV)-1 in BALB/c mice (38); supraciliary inoculation of murine cytomegalovirus in euthymic mice (1); intrastromal inoculation of VZV in Guinea pigs (29); and intrastromal and subconjunctival injection of simian varicella virus in African green monkeys (25). Those animal models have two major limitations: 1) the unnatural route of inoculation, and 2) the lack of host–pathogen co-evolution; thus the actual disease process may not be reflected. In this work, we show that MD-induced ocular lesions recapitulate several features of VZV- and HSV-induced ocular disease. In addition, MDV shares close biologic and genetic similarity to both, especially with VZV (27). Experimental model of MDV infection has been amply studied (43). There are numerous, well-characterized pathogenic and vaccine MD strains, as well as chicken lines, that could be very useful for studying the spectrum of ocular lesions. A major advantage of using MD as a model for herpesvirus-induced ocular lesions is that chickens are the natural host for MDV; therefore, we can study the early stages of infection, as well as immune responses, without intraocular inoculation. The only drawback might be the avascular retina of the chicken. However, the highly vascular choroid layer and the pecten appear to substitute well for the avascular retina, and the chicken eye has served as a useful model to study various degenerative retinal disorders of humans (4,39).

This work contributes to our understanding of the pathogenesis of MDV-induced ocular lesions in chickens, and it might serve as a model for understanding the pathogenesis of the ocular lesions induced by human herpesviruses. Our results show that ocular lesions started very early after infection and progressed in severity and distribution with time. Early ocular lesions resemble inflammatory lesions described in the brain and in other locations (3,16,31). However, late lesions differed from the neoplastic lesions described in other locations and have features common with type B lesions in the peripheral nerves. In addition, we have demonstrated that the protection patterns conferred by vaccines differ for early and late lesions, and that only protection of late lesions depends on the efficacy of the vaccine used.

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